invitrogen™

# Lipofectamine™ 2000

**Cat. No. 11668-027** **Size: 0.75 ml**
**Cat. No. 11668-019** **Size: 1.5 ml**
**Cat. No. 11668-500** **Size: 15 ml**
**Store at +4ºC (do not freeze)**

## Description

Lipofectamine™ 2000 is a proprietary formulation for the transfection of nucleic acids (DNA and RNA) into eukaryotic cells providing the following advantages:

- Highest transfection efficiency in many cell types and formats (*e.g.* 96-well). Refer to the Cell Lines database at www.invitrogen.com for a list of cell types successfully transfected.
- Nucleic acid-Lipofectamine™ 2000 complexes can be added directly to cells in culture medium, in the presence or absence of serum.
- It is not necessary to remove complexes or change/add medium after transfection, but complexes may be removed after 4-6 hours.

## Important Guidelines for Transfection

- Use the procedure on page 2 to transfect cells with short interfering RNA (siRNA) or Stealth™ RNAi.
- Use the procedure on page 3 to transfect cells with plasmid DNA.
- We recommend Opti-MEM® I Reduced Serum Medium (Cat. No. 31985-062) to dilute Lipofectamine™ 2000 and nucleic acids before complexing.
- **Do not** add antibiotics to media during transfection as this causes cell death.
- Maintain the same seeding conditions between experiments.
- Test serum-free media for compatibility with Lipofectamine™ 2000 since some serum-free formulations (*e.g.* CD293, SFM II, VP-SFM) may inhibit cationic lipid-mediated transfection.

**Note:** For more tips for your RNAi experiment, refer to "Seven Steps to RNAi Success". This manual is available from www.invitrogen.com/rnai or Technical Service, as are cell-type specific RNAi transfection protocols (see "RNAi protocols".)

## Quality Control

Lipofectamine™ 2000 is tested for absence of microbial contamination with blood agar plates, Sabaraud dextrose agar plates, and fluid thioglycolate medium, and functionally by transfection of CHO-K1 cells with a reporter plasmid.

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## Stealth™ RNAi or siRNA Transfection

Use this brief procedure to transfect **Stealth™ RNAi** or **siRNA** into mammalian cells in a **24-well format**. For other formats, see **Scaling Up or Down Transfections** (page 4). All amounts and volumes are given on a per well basis. Use this procedure as a starting point; optimize transfections as described in **Optimizing Stealth™ RNAi or siRNA Transfection**, especially if you are transfecting a mammalian cell line for the first time.

1. One day before transfection, plate cells in 500 µl of growth medium without antibiotics such that they will be **30-50% confluent** at the time of transfection. **Note:** Transfecting cells at a lower density allows a longer interval between transfection and assay time, and minimizes the loss of cell viability due to cell overgrowth.
2. **For each transfection sample**, prepare oligomer-Lipofectamine™ 2000 complexes as follows:
   a. Dilute 20 pmol Stealth™ RNAi or siRNA oligomer in 50 µl Opti-MEM® I Reduced Serum Medium without serum (final concentration of RNA when added to the cells is 33 nM). Mix gently.
   b. Mix Lipofectamine™ 2000 gently before use, then dilute 1 µl in 50 µl Opti-MEM® I Reduced Serum Medium. Mix gently and incubate for 5 minutes at room temperature. **Note:** Proceed to Step c within 25 minutes.
   c. After the 5-minute incubation, combine the diluted oligomer with the diluted Lipofectamine™ 2000. Mix gently and incubate for 20 minutes at room temperature (solution may appear cloudy).
3. Add the oligomer-Lipofectamine™ 2000 complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.

Incubate the cells at 37°C in a $CO_2$ incubator for 24-96 hours until you are ready to assay for gene knockdown. Medium may be changed after 4-6 hours.

## Optimizing Stealth™ RNAi or siRNA Transfection

To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions by varying RNA and Lipofectamine™ 2000 concentrations. Test 10-50 pmol RNA and 0.5-1.5 µl Lipofectamine™ 2000 for 24-well format. Depending on the nature of the target gene, transfecting cells at higher densities may also be considered when optimizing conditions.

## Plasmid DNA Transfection

Use the following procedure to transfect DNA into mammalian cells in a **24-well format**. For other formats, see **Scaling Up or Down Transfections** (page 4). All amounts and volumes are given on a per well basis. Prepare complexes using a DNA (µg) to Lipofectamine™ 2000 (µl) ratio of 1:2 to 1:3 for most cell lines. Transfect cells at high cell density for high efficiency, high expression levels, and to minimize cytotoxicity. Optimization may be necessary (see **Optimizing Plasmid DNA Transfection**, page 4).

1. **Adherent cells:** One day before transfection, plate 0.5-2 x $10^5$ cells in 500 µl of growth medium without antibiotics so that cells will be **90-95% confluent** at the time of transfection.

   **Suspension cells:** Just prior to preparing complexes, plate 4-8 x $10^5$ cells in 500 µl of growth medium without antibiotics.

2. **For each transfection sample**, prepare complexes as follows:
   a. Dilute DNA in 50 µl of Opti-MEM® I Reduced Serum Medium without serum (or other medium without serum). Mix gently.
   b. Mix Lipofectamine™ 2000 gently before use, then dilute the appropriate amount in 50 µl of Opti-MEM® I Medium. Incubate for 5 minutes at room temperature. **Note:** Proceed to Step c within 25 minutes.
   c. After the 5 minute incubation, combine the diluted DNA with diluted Lipofectamine™ 2000 (total volume = 100 µl). Mix gently and incubate for 20 minutes at room temperature (solution may appear cloudy). **Note:** Complexes are stable for 6 hours at room temperature.
3. Add the 100 µl of complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.
4. Incubate cells at 37°C in a $CO_2$ incubator for 18-48 hours prior to testing for transgene expression. Medium may be changed after 4-6 hours.
5. **For stable cell lines:** Passage cells at a 1:10 (or higher dilution) into fresh growth medium 24 hours after transfection. Add selective medium (if desired) the following day.

## Optimizing Plasmid DNA Transfection

To obtain the highest transfection efficiency and low cytotoxicity, optimize transfection conditions by varying cell density as well as DNA and Lipofectamine™ 2000 concentrations. Make sure that cells are greater than 90% confluent and vary DNA (µg): Lipofectamine™ 2000 (µl) ratios from 1:0.5 to 1:5.

## Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of Lipofectamine™ 2000, nucleic acid, cells, and medium used in proportion to the relative surface area, as shown in the table. With automated, high-throughput systems, a complexing volume of 50 µl is recommended for transfections in 96-well plates. **Note:** You may perform rapid 96-well plate transfections by plating cells directly into the transfection mix. Prepare complexes in the plate and directly add cells at twice the cell density as in the basic protocol in a 100 µl volume. Cells will adhere as usual in the presence of complexes.

| Culture vessel | Surf. area per well[1] | Shared reagents | | DNA transfection | | RNAi transfection | |
|---|---|---|---|---|---|---|---|
| | | Vol. of plating medium | Vol. of dilution medium[2] | DNA | Lipofectamine™ 2000 | RNA | Lipofectamine™ 2000 |
| 96-well | 0.3 $cm^2$ | 100 µl | 2 x 25 µl | 0.2 µg | 0.5 µl | 5 pmol | 0.25 µl |
| 24-well | 2 $cm^2$ | 500 µl | 2 x 50 µl | 0.8 µg | 2.0 µl | 20 pmol | 1.0 µl |
| 12-well | 4 $cm^2$ | 1 ml | 2 x 100 µl | 1.6 µg | 4.0 µl | 40 pmol | 2.0 µl |
| 6-well | 10 $cm^2$ | 2 ml | 2 x 250 µl | 4.0 µg | 10 µl | 100 pmol | 5 µl |
| 60-mm | 20 $cm^2$ | 5 ml | 2 x 0.5 ml | 8.0 µg | 20 µl | 200 pmol | 10 µl |
| 10-cm | 60 $cm^2$ | 15 ml | 2 x 1.5 ml | 24 µg | 60 µl | 600 pmol | 30 µl |

[1] Surface areas may vary depending on the manufacturer.
[2] Volumes of dilution medium in Step 2a & 2b of DNA or RNAi transfection protocols.

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